SCIENTIFIC LETTERS

Interleukin 18 in acute myocardial infarction

Interleukin 18 (IL-18), which is a recently cloned cytokine synthesised by Kupffer cells, has been shown to activate macrophages.[1] Macrophages and T cells have been reported to be activated in patients with acute myocardial infarction (MI).[2] IL-18 is postulated to play a role in the development of myocardial dysfunction through activation of immune cells. However, the circulating concentration of IL-18 has not been reported in patients with acute MI.

To elucidate the changes in the release of IL-18 in patients with coronary artery disease, we examined the correlation between IL-18 concentrations and the serum activities of myocardial enzymes, estimating myocardial necrosis or atrial natriuretic peptide (ANP). Our goal was to determine whether measurement of IL-18 could be used as a new indicator of myocardial damage in patients with acute MI.

We evaluated 24 patients with acute MI (19 men and five women aged 39–79 years). Patients diagnosed with acute MI had to have been admitted within six hours of the onset of symptoms. The diagnosis of acute MI was based on the presence of typical chest pain which persisted for at least 30 minutes, ST segment elevations > 0.2 mV in at least two contiguous leads, and an increase in serum creatine kinase activity to twice the upper limit of normal (> 250 IU/l). The diagnosis of congestive heart failure was based on a history of dyspnoea and symptomatic exercise intolerance with signs of pulmonary congestion or peripheral oedema at clinical examination, and following usual investigations. As a control group, we included 12 age and sex matched patients who had no evidence of coronary stenosis or coronary spasm on coronary angiography. No patient had evidence of septicaemia, collagen vascular disease, advanced liver disease, renal failure, malignancy or infectious disease.

Blood was sampled immediately after admission and at 1, 2, 3, 6, and 9 hours after admission and then every 12 hours until five days after admission. Blood was drawn from the antecubital vein into silicone tubes (4.5 ml of blood and 0.5 ml of 0.13 M/l sodium citrate), and centrifuged for 15 minutes at 1600 g at 40°C. The plasma was stored at −80°C until assayed. Human IL-18 was measured by an enzyme linked immunosorbent assay (ELISA) developed by Hayashibara Inc, Okayama, Japan.[3] Serum creatine kinase-MB (CK-MB), myoglobin, lactate dehydrogenase (LDH) activities, and leucocyte counts were measured by an autoanalyser. Data are expressed as mean (SD). Values for patients with acute MI and the control group were compared using an unpaired *t* test. The minimum concentration of IL-18 is 10 pg/ml. Correlations between IL-18 concentrations and other parameters were analysed by linear regression. A probability value of $p < 0.05$ was considered significant.

Plasma IL-18 concentrations were significantly higher in patients with acute MI than in controls (46 (16) pg/ml and 26 (10) pg/ml, respectively, $p < 0.05$). IL-18 and other indicators of acute MI at the time of maximal rise and the peak titre are shown in table 1. The maximal rise of IL-18 was later than that of myoglobin and CK-MB, but significantly earlier than that of LDH. The peak IL-18 concentration in patients with acute MI correlated with the serum activities of CK-MB, aspartate transaminase (AST), and LDH ($r = 0.54$, $p < 0.05$; $r = 0.48$, $p < 0.01$; and $r = 0.55$, $p < 0.05$, respectively). The circulating IL-18 was positively correlated with plasma concentrations of ANP.

This study shows that circulating concentrations of IL-18 increase in patients with acute MI. We hypothesise that higher concentrations of IL-18 may be a new marker of cardiac damage in the development of acute MI.

Based on our findings, the clinical role of IL-18 can be hypothesised. IL-18 induces IFN-γ gene expression in activated T cells and macrophages, and also induces apoptosis in Fas-expressing activated T cells.[4] Thus, increases in IL-18 in patients with acute MI induces apoptosis in activated T cells, leading to persistent T cell mediated reaction to self antigens in myocytes.[5] On the other hand, apoptosis is the major form of myocardial damage after infarction, since the expression of Fas is enhanced in the ischaemic myocytes.[6] Therefore, increases in serum IL-18 concentrations may induce apoptosis in myocytes, leading to persistent myocardial damage in acute MI.

In conclusion, increased secretion of IL-18 occurs in patients with acute MI and correlates with the severity of myocardial damage. In the clinical setting, our results suggest that measurement of IL-18 may be a new marker of cardiac damage in patients with acute MI.

YUKIHIRO SETA
TSUGIYASU KANDA*
TORU TANAKA
MASASHI ARAI
KENICHI SEKIGUCHI
TOMOYUKI YOKOYAMA
MASASHI KURIMOTO†
JUN'ICHI TAMURA*
MASAHIKO KURABAYASHI
*Second Department of Internal Medicine, *Department of General Medicine, Department of Laboratory Medicine, Gunma University School of Medicine, 3-39-15, Showa-machi, Maebashi 371, Japan †Hayashibara Biochemical Laboratories Inc, 675-1, Fujisaki, Okayama, 702-8006 Japan*
kanda@showa.gunma-u.ac.jp

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Table 1 IL-18 and indicators of acute myocardial infarction

Marker	*Initial rise (hours)*	*Maximal rise (hours)*	*Maximal value*
IL-18	2.2 (0.9)	13 (8)	82 (17) pg/ml
Leucocytes	4.2 (1.2)	10 (3)	12900 (2000) /mm^3
Myoglobin	4.0 (1.3)	5.2 (1.2)	465 (215) ng/ml
CK-MB	4.1 (1.2)	11.0 (3.7)	16.5 (1.9) IU/L
LDH	6.2 (1.7)	46 (19)	1723 (455) IU/L

Exercise induced myocardial ischaemia does not cause increase in C-reactive protein concentration

High plasma concentrations of C-reactive protein (CRP), which are predictive of a poor prognosis in patients with acute coronary syndromes,[1] might reflect an inflammatory pathogenetic component. It is well known, however, that short periods of ischaemia are powerful inflammatory stimuli sufficient to induce the synthesis of acute phase reactants.[2] Therefore, the acute phase response in acute coronary syndrome may simply reflect the extent and severity of myocardial ischaemia. To establish whether myocardial ischaemia is an adequate stimulus to induce an increase in CRP, its plasma concentrations were evaluated in a group of patients with chronic stable angina and normal baseline values of CRP in whom myocardial ischaemia was induced by exercise.

Fifteen patients (10 men, mean (SD) age 60 (9) years) with chronic stable angina and reproducible positive exercise testing for myocardial ischaemia participated in this study. No patient had suffered a previous myocardial infarction and mean left ventricular ejection fraction was 62 (5)%. All patients had at least one critical stenosis in the proximal two thirds of one major epicardial coronary artery. The control group consisted of nine healthy subjects (six men, mean age 61 (9) years) without evidence of ischaemic heart disease and normal physical examination, rest ECG, and echocardiogram. After pharmacological washout, treadmill exercise testing was performed following the modified Bruce protocol. The level of the ST segment, 80 ms after the J point, was calculated after signal averaging by means of a computer assisted system in all 12 leads.

For CRP, venous blood samples were obtained immediately before (T_0), at the end of the exercise test (T_1), and 6 (T_2), 24 (T_3), and 48 (T_4) hours after the exercise test. CRP concentration was immunologically determined by immunoturbidimetric method (Roche Unimate 3 CRP, Milan, Italy). The normal upper reference value for CRP with this method is up to 5 mg/l. Data on CRP are expressed as median values and interquartile ranges and were analysed by using two way analysis of variance (ANOVA) for repeated measures. Differences between groups were considered to be significant at a probability value of $p < 0.05$.

All patients with chronic stable angina had positive exercise tests and reached ST segment depression > 2.0 mm with a mean (SD) value of 2.9 (0.3) mm at 80 (13)% of the predicted heart rate. Ten patients complained of typical chest pain during exercise testing. All controls had negative exercise test and none experienced chest pain. The mean value of ST segment depression was 0.53 (0.18) at 96 (3)% of the predicted heart rate.

Table 1 C-reactive protein plasma concentrations before and after exercise testing at the different sampling points

Sample	Stable angina	95% CI	Controls	95% CI
T_0	3.28 (2.6–3.9)	2.7 to 3.8	2.04 (1.6–3.4)	1.7 to 3.3
T_1	3.46 (2.8–4.0)	2.9 to 4.0	2.34 (1.4–3.7)	1.6 to 3.5
T_2	3.36 (2.6–4.1)	2.6 to 4.0	2.25 (1.8–3.4)	1.9 to 3.4
T_3	3.18 (2.4–3.9)	2.3 to 3.7	2.25 (1.5–3.3)	1.5 to 3.2
T_4	2.98 (2.3–3.8)	2.3 to 3.5	3.24 (1.9–3.2)	2.0 to 3.4

Values are expressed in mg/l and represent medians and interquartile ranges. Differences are not significant. See text for explanation of T_0–T_4. CI, confidence interval.

Baseline values of CRP were similar in patients and controls (table 1). At the end of exercise testing and 6, 24, and 48 hours after the testing the values of CRP did not change significantly in both groups.

CRP is an acute phase reactant and represents a sensitive marker for underlying systemic inflammation.[3] Recently, its prognostic role in a variety of cardiovascular conditions has been repeatedly emphasised. Baseline concentrations of CRP in apparently healthy persons, in subjects with cardiovascular risk factors, and in patients with stable angina represent an independent risk factor for future cardiovascular events. Furthermore, high concentrations of CRP in patients with unstable angina and myocardial infarction are associated with a worse prognosis independently of the extent and severity of myocardial ischaemia or cell injury. Finally, persistently raised plasma concentrations of CRP after coronary surgery have been shown to be predictive of postoperative complications, and in patients with coronary artery disease undergoing successful coronary artery angioplasty or stent implantation, pre- and postprocedural higher plasma concentrations of CRP are independent predictors of cardiac events at follow up.[4]

On the basis of this clinical evidence it is assumed that the increase in CRP is not secondary to the extent and severity of myocardial ischaemia, but it may be an independent marker of the inflammatory component involved in the pathogenesis of acute coronary syndromes. Many experimental studies, however, have shown that short periods of ischaemia and reperfusion are powerful proinflammatory stimuli capable of inducing leucocyte and complement activation, cytokine production, and acute phase protein synthesis.[2] The increase in acute phase reactant may therefore simply be the biochemical marker of recurrent myocardial ischaemia–reperfusion episodes. Yet, in this study, carried out in patients with chronic stable angina and normal plasma concentrations of CRP, severe exercise induced myocardial ischaemia failed to affect CRP serum concentrations, thus indicating that myocardial ischaemia "per se" is not an adequate stimulus to cause an increase in CRP. Thus, the increase of CRP serum concentrations observed in patients with acute coronary syndromes is unlikely be caused by myocardial ischaemia, but it is more likely to represent a marker of the inflammatory process involved in the pathogenesis of myocardial ischaemia.

A potential flaw of our study is that the duration and severity of transient exercise induced myocardial ischaemia might not have been sufficient to produce an adequate stimulus to induce the production of CRP. Yet, in a previous study, no increase in CRP plasma concentrations was observed in patients with variant angina despite the longer duration of the single ischaemic episodes (> 10 minutes) and total ischaemic burden (> 40 minutes).[5] Furthermore, although the number of patients and controls was small, the statistical power of the study in the assessment of CRP changes within and between groups was sufficiently high (> 80% for pairwise comparisons); thus it is unlikely that significant changes in CRP were not detected.

In conclusion, the results of the present study, showing that exercise induced severe myocardial ischaemia does not induce an increase in CRP, support the concept that myocardial ischaemia "per se" is not an adequate stimulus to cause an increase in CRP serum concentrations, and that the increase observed in patients with acute coronary syndromes is unlikely to be related to myocardial ischaemia but is rather a marker of an underlying inflammatory process which may contribute to the pathogenesis of myocardial ischaemia.

A GASPARDONE
M PERINO
A S GHINI
F TOMAI
F VERSACI
I PROIETTI
F CREA
Divisione di Cardiochirurgia, Università di Roma Tor Vergata, European Hospital, via Portuense 700, 00149 Rome, Italy
gaspardone@tin.it

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